

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of : **Confirmation No. 4315**
Jan BARNIKOW et al. : Attorney Docket No. 2006_1322A
Serial No. 10/591,159 : Group Art Unit 1751
Filed October 3, 2006 : Examiner Yong D. Pak
MUTANTS OF O6-ALKYLGUANINE-DNA
ALKYLTRANSFERASE : **Mail Stop: AMENDMENT**

SUPPLEMENTAL RESPONSE TO RESTRICTION REQUIREMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the Office Communication dated January 21, 2009, and in further response to the Restriction/Species Election Requirement dated October 7, 2008, Applicants elect the sequence in Example 8, Table 1, second sequence. Applicants note that the elected species having the following mutations vs. human AGT:

Lys32Ile, Leu33Phe, Cys62Ala, Gln115Ser, Gln116His, Lys125Ala, Ala127Thr, Arg128Ala, Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser, Cys150Gly, Ser151Gly, Ser152Asp, Ala154Asp, Asn157Gly, Ser159Glu, truncation at 182.

(Underlining means double mutations resulting in the underlined mutation)

has the following properties:

- (a) no detectable DNA interaction;
- (b) localization of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus;
- (c) at least 10-fold improved expression yield as soluble protein and improved stability in *E. coli*;

- (d) improved stability under oxidizing conditions;
- (e) at least six-fold improved stability within cells after reaction with a substrate;
- (f) improved stability outside cells before and after reaction with a substrate;
- (g) at least four-fold increased *in vitro* solubility;
- (h) 45-fold higher reactivity against O⁶-alkylguanine substrates;
- (i) no detectable reactivity against DNA-based substrates; and
- (j) at least 10-fold reduced reactivity against N⁹-substituted O⁶-alkylguanine substrates.

The elected species reads on claims 1-7, 9-13, 16, 17, 19, 20, 24 and 37.

In combination with the Response to Restriction/Species Election Requirement dated November 3, 2008, Applicants believe that a full response to the Restriction/Species Election Requirement dated October 7, 2008 has now been provided.

Favorable action on the merits is now requested.

Respectfully submitted,

Jan BARNIKOW et al.

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January 30, 2009